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THE DIVERSITY OF SOME PHENOLOGICAL FEATURES IN BLUEBERRY CULTIVARS (*VACCINIUM CORYMBOSUM* L.) GROWN IN BANAT AREA, ROMANIA

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Abstract

This paper's aim was to investigate the adaptation to the environmental conditions of south-western Romania, Banat region (45°25'48"N 21°34'55"E) in terms of flowering and ripening seasons, of 3 commercial blueberry (V. corymbosum) cultivars of North American origin in order to examine and determine the levels of genetic difference. This study was carried out during the growing season in a blueberry plantation, for 'Duke', 'Hannah's Choice' and 'Elliott' varieties. In the study, phenological traits were recorded using the BBCH phenological scale for blueberry and the observation of phenotypic data was recorded as the number of days from January 1st for statistical analyses. Cultivars were found to have quite different characteristics in terms of phenological traits. The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV = 14.08%), while the lowest CV was given by bud break (53 BBCH) (CV = 5.70%). It was found that the differences in phenology recorded between cultivars are due to the different accumulation of the amount of temperature required for the development of a certain phenophase, depending on the cultivar's requirements, knowing that the temperature factor is crucial for exiting from dormancy, flowering and fruit ripening.

Key words*: blueberry, diversity, phenology.*

INTRODUCTION

Cultivated blueberry (*Vaccinum corymbosum*), native to North America, was introduced to Romania in the late 1960s and the fast upward trend of blueberry establishment in Romania is motivated by the growing market demand for fresh fruit consumption and the profit incentive compared with other fruit produced in the orchards (Asanica et al., 2017). Blueberry cultivation is of growing interest due to the nutritional qualities of fruits and the therapeutic virtues of both fruits and leaves (Moze et al., 2011; Kostenko et al., 2020; Tundis et al., 2021). One of the most important aspects for a successful expansion of the blueberry crop is the selection of cultivar, which basically depends on knowledge about their adaptation to local conditions (Campa & Ferreira, 2018; Bădescu et al., 2016; Peticila et al., 2009; Merca & Cosmulescu, 2020). Environmental factors, different from one year to another, or from one area to another, do require the different development of vegetation phenophases in fruit plants (Cosmulescu and Ionescu, 2018, 2021; Cosmulescu et al., 2010; Asanica et al., 2020). The registration of the landmark stages in the fruit plants is necessary in order to have useful data regarding the periods of their development, and also for the establishment of assortments adapted to the cultivation areas. Vegetation stages of fruit trees are of high economic importance because they have a direct impact on fruit production (Chmielewski, 2004), which shows the importance of knowledge of fruit trees phenology. Findings of Zverko et al. (2014) indicate that the increase in temperature has a significant influence on the onset of spring phenophases in some fruit species. Systematic long-term monitoring of phenophases provides an opportunity to estimate changes in the onset or end of phenophases, which allows the assessment of the influence of climate change on nature (Bauer et al., 2014). Monitoring of environmental conditions can be crucial for

farmers wishing to implement management practices at specific stages of crop development (Kirk & Isaacs, 2012). The aim of this paper was to investigate the adaptation to the environmental conditions of south-western Romania, Banat region (45°25′48″N 21°34′55″E) in terms of flowering and ripening seasons, of 3 commercial blueberry cultivars of North American origin, in order to examine the levels of genetic difference.

MATERIALS AND METHODS

This study was conducted during the vegetation period 2020 and 2021 in a blueberry plantation (*V. corymbosum*). Three blueberry cultivars were taken under study: 'Duke' (USA origin), 'Hannah's Choice' (USA origin), 'Elliott' (USA origin). 'Duke' blueberry is an early-season cultivar with attractive fruit of medium to large size, light blue coloured.

'Hannah's Choice' is a vertical shrub with pink flowers, blooms early and may be susceptible to late frost damage (Ehlenfeldt et al., 2005). 'Elliott' cultivar blooms late to avoid late spring frosts, it is cold-resistant, ripens in August (Ehlenfeldt, 2003).

In order to determine the genetic diversity, phenological traits were identified on a total of 75 plants for each cultivar. The experiment was organized in Gherteniş (45°25′48″N 21°34′55″E), Caraş-Severin County, Banat region, located in south-western Romania. In the study, phenological traits were recorded using the BBCH phenological scale for blueberry as described by Longstroth (2008). Twelve main growth stages have been described (Table 1, Figure 1). The observations were recorded and converted to Julian Dates (number of days after January 1) with the aim to conduct a quantitative analysis (Cosmulescu et al., 2022). The data were statistically analyzed using descriptive statistics and correlation by using Statistical Package Programme - Data Analysis.

Table 1. Definitions used in the determination of phenological traits - flower bud*

BBCH scale/Traits	Description
51/Bud swell	First sign of growth as plant growth begins in the spring. Visible swelling of the flower buds; outer bud scales begin to separate at the tip revealing paler interior bud scales. This bud stage can usually tolerate cold temperatures of -12 to -9°C.
53/Bud break	Flower buds open and the individual flowers can be seen between the bud scales. Can tolerate cold temperatures of about -7°C.
55/Tight cluster	Individual flowers are distinguishable in the flower cluster. This bud stage can tolerate -7 to -5°C.
57/Early pink bud	Expanding flowers are readily visible and have separated. The pink corolla tubes (petals) are short and closed. This bud stage can tolerate -5 to -4°C.
59/Late pink bud	Individual flowers fully developed. Expanded corollas are now white but still closed. This bud stage can tolerate -4.4 to -2.8°C.
65/Full bloom	Most of the flowers on the bush have opened. The bloom stages can tolerate -2.2°C.
67/Petal fall	The corolla tubes are falling off the flowers, revealing small green fruit. This is the most vulnerable stage to freeze injury. Damage can occur at 0°C.
71/Early green fruit	Small green berries are expanding. Fruit in the cluster varies from large to small pea-sized. Early fruit growth is by cell division.
78/Late green fruit	Growth of large fruit slows. Fruit becomes pale green. Exposed fruit may develop a red blush.
81/Fruit colouring	Oldest, largest fruit in the cluster begin to change colour from green to pink to blue. Fruit begins to soften. Cell division has stopped and fruit growth is by cell expansion.
85/25% Blue	25 percent of the berries are ripe. 25% blue often coincides with first hand harvest of ripe berries.
87/75% Blue	Blueberries are picked several times as the fruit ripens with 2 to 5 pickings. Often the first harvest is by hand and then later by machines that shake berries off the bush. 75% blue often coincides with the first of 2 machine harvests in the field.

*Source: https://www.canr.msu.edu/blueberries/growing_blueberries/growth-stages

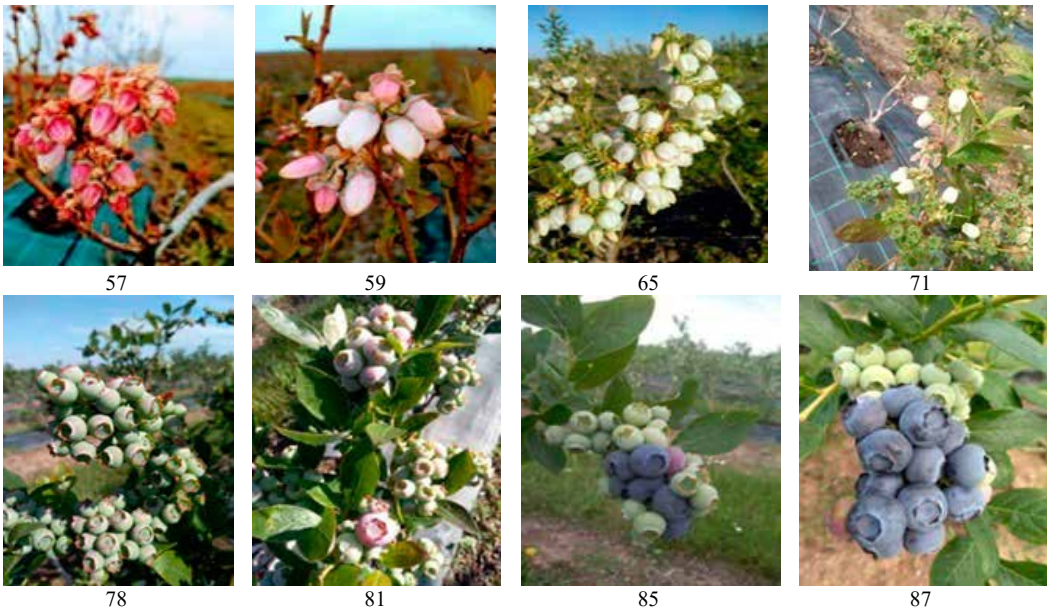

Figure 1. Blueberry phenological stages according to the BBCH scale (original pictures)

RESULTS AND DISCUSSIONS

Statistical data on phenological features of the analysed blueberry varieties are presented in the Table 2. We determined a large phenological diversity in our blueberry cultivars. Cultivars were found to have quite different characteristics in terms of phenological traits.

The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV=14.08%), while the lowest CV was given by bud break (53 BBCH) (CV= 5.70%) (Table 1).

The summary findings of bud swell (51) bud break (53), tight cluster (55), early pink bud (57), late pink bud (59), full bloom (65), petal fall (67), early green fruit (71), late green fruit (78), fruit colouring (81), 25% fruit blue (85) and 75% fruit blue (87) dates of genotypes are presented in Table 2.

According to the values obtained for the coefficient of variability, it is found that the phenological changes in the population were homogeneous (CV<10%) and relatively homogeneous (10%<CV<20%).

Table 2. Descriptive statistics of the number of days (Julian date) necessary for the development of the main spring phenological traits in blueberries

TRAITS	UNIT	MIN	MAX	MEAN	SD	CV%
51/Bud swell	Day	39	60	53	3.20	6.03
53/Bud break	Day	60	88	70.33	4.01	5.70
55/Tight cluster	Day	70	101	82.5	5.39	6.53
57/Early pink bud	Day	79	117	97.66	13.76	14.08
59/Late pink bud	Day	94	121	106	10.21	9.63
65/Full bloom	Day	104	134	119.83	11.49	9.58
67/Petal fall	Day	114	140	127.33	10.32	8.10
71/Early green fruit	Day	121	152	134.5	12.14	9.02
78/Late green fruit	Day	130	166	145.33	13.50	9.28
81/Fruit colouring	Day	139	181	154.66	16.59	10.72
85/25% Blue	Day	160	205	179.16	19.27	10.75
87/75% Blue	Day	175	222	190.83	20.62	10.80

The lowest coefficient of variation was obtained for phenophase 53 BBCH/Bud break (CV%=5.70) while the largest for 57 BBCH/Early pink bud phenophase (CV%=14.08).

Analyzing the limits of variation for each phenophase, it is found that the differences between the years were quite large, ranging between 21 days for bud swell phenophase (BBCH 51) and 47 days for / 75% blue phenophase (BBCH 87) (Table 1).

Lower temperatures, with values around 0° C or lower, both in the air and on the soil, which favour hoar-frosts or late frosts, after March 20, represent a major risk for all crops. In the present case study, both over the spring of 2020 and over the year 2021, after successive days with normal values for the end of March and the beginning of April, days followed with temperatures below 0°C both in the air and on the soil. The high risk was the fact that there were consecutive days with negative values. From a calendar point of view, the early pink bud phenophase (57 BBCH) occurred between March 20 and April 29, i.e. 79-117 days from January 1 (Table 1). Over this period, the minimum air temperatures had values between -2.7°C (March 20, 2021) and 2°C (March 22, 2020), and the soil temperatures between -4.2°C (March 20, 2021) and 3°C (April 6, 2020). The negative effect of these temperatures was observed in the 'Hanna's Choice' cultivar, when 7.6% of buds were affected, with repercussions on crops. In this bud stage (57 BBCH), according to Longstroth (2008), it can tolerate temperature values of -5 to -4°C. Late pink bud (59) was evaluated on April 5 for 'Hannah's Choice' cultivars in 2020 year, and on May 3 for 'Elliott' cultivars. The difference between January 1 and the late pink bud (59) was from 94 to 122 days (Table 2). From a calendar standpoint, the full bloom phenophase (65 BBCH) ran from April 15 ('Hannah's Choice', 2020) to May 16 ('Elliott', 2021). The flowering is a very important parameter because of the possible damage to flowers by late spring frost (Sterne & Liepniece, 2010). The full bloom (65 BBCH) stages can tolerate -2.2°C according to Longstroth (2008). In the research area, in this phenophase, the minimum recorded temperatures were between 5.5 and 20.8°C, which did not create problems, from a climatic point of view, for this phenophase. The phenophase of early green fruit (71 BBCH) was noticed starting with May 2 ('Hannah's Choice', 2020) and a month later ('Elliott', 2021) and occurs at 121-152 days from January 1 and depends on the cultivar, weather and the plant's vigour. Fruits continue to grow even after the fruits turn blue by accumulating water in the tissues. Since at the end of fruit ripening the water needs of the plant increase, in order to obtain quality fruits, it is very important to properly irrigate the crop during this critical period. The time of fruit ripening (25% blue, 85 BBCH) fluctuated from 160 to 205 days, by cultivar and climatic year. This phenophase occurred first in the 'Duke' cultivar (June 10, 2020) and in the latest in 'Elliott' (July 25, 2020). With regard to phenophase 87 BBCH (75% blue), the results obtained indicated that the length of the period was from 1 year to this one (87 BBCH) in cultivars of so it ranged from 175 to 222 days (Table 1). 'Hannah's Choice' ripens at approximately the same time as 'Duke', when 'Eliott' is in the fruit colouring phenophase (81 BBCH). According to Sterne & Liepniece (2010), the best blueberry yields are usually obtained by fruit ripening very early or very late in the season. The choice of the 3 cultivars, from this point of view, is an advantage. Correlation coefficient was used to determine the associations between the phenological traits (Table 3). Values of the correlation coefficient for trait pairs were significant and positive in most cases. We determined a very significant correlation between early green fruit (71 BBCH) and late pink bud (59 BBCH) (r = 0.994), between early green fruit (71 BBCH) and fruit colouring (81 BBCH) (r = 0,977), between late pink bud (59 BBCH) and fruit colouring (81 BBCH) (r = 0.977), between 65 / full bloom and 71 and early green fruit (71 BBCH), late green fruit (78 BBCH) (r = 0.967), between 25% blue (85 BBCH) and 75% blue (87 BBCH) (r = 0.986) etc. Our data indicated that the first phenophases of spring (late pink buds - 59 BBCH; early green fruits - 71 BBCH) were highly correlated with later BBCH stages (fruit coloring - 81 BBCH; 25% blue - 85 BBCH; 75% blue - 87 BBCH), which suggests a potential relationship between physiological

resting state and apparent morphological changes in blueberry flower buds. Significant correlations between phenological traits were determined on blueberry cultivars by Campa & Ferreira (2018). Negative significant correlations were observed between flowering season and days to flowering, flowering season and days to the end of flowering, and harvesting season and days to flowering in the same study by Campa & Ferreira (2018).

Table 3. Correlation coefficient between the phenological traits of blueberry varieties*

	57 BBCH	59 BBCH	65 BBCH	71 BBCH	78 BBCH	81 BBCH	85 BBCH	87 BBCH
57 BBCH	1							
59 BBCH	0.972***	1						
65 BBCH	0895	0.951***	1					
71 BBCH	0.943***	0.994***	0.967***	1				
78 BBCH	0.893	0.955***	0.966***	0.969***	1			
81 BBCH	0.937***	0.977***	0.916***	0.977***	0.969***	1		
85 BBCH	0.818	0.896	0.817	0.906***	0.803	0.855	1	
87 BBCH	0.855	0.909***	0.791	0.905***	0.806	0.880	0.986***	1

***p<0,001

CONCLUSIONS

In conclusion, cultivars were found to have quite different characteristics in terms of phenological traits. The highest coefficient of variation belonged to early pink bud (57 BBCH) (CV = 14.08%), while the lowest CV was given by bud break (53 BBCH) (CV = 5.70%). It was found that the differences in phenology recorded between cultivars are due to the different accumulation of the amount of temperature required for the development of a certain phenophase, depending on the cultivar's requirements, knowing that the temperature factor is crucial for exiting from dormancy, flowering and fruit ripening. Our results indicate that blueberry varieties have shown a high degree of phenotypic plasticity to respond to gradual changes in environmental conditions.

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